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Determination of Propionylpromazine Hydrochloride in Formulation Matrixes Using Reversed-Phase Ion-Pair Small Bore Liquid Chromatography

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Propionylpromazine hydrochloride (PPZHCl) has been investigated for use with leghold traps to reduce the amount of self-inflicted trauma experienced by animals restrained by these traps. Three types of PPZHCl formulations made with Karo dark syrup, K-Y Jelly, and Vaseline were used in 2 types of tranquilizer trap devices (TTDs). A reversed-phase ion-pair liquid chromatography (LC) method using a small bore C_{18} column was used to: (*1*) determine the purity of the PPZHCl material used in these formulations, and (*2*) to determine the resulting PPZHCl content of each formulation. Analyte quantitation was done using UV absorption at 280 nm. Regression analysis of calibration standard solutions indicated a linear and directly proportional relationship between analyte response and PPZHCl concentration over the range evaluated. Recovery data from: (*1*) Vaseline formulations containing 38.8, 16.2, and 8.78% PPZHCl were 104, 92.9, and 90.2%, respectively, (*2*) Karo dark syrup formulations containing 26.5, 18.1, and 10.3% PPZHCl were 97.7, 99.3, and 106%, respectively, and (*3*) K-Y Jelly formulations containing 33.0, 23.5, and 13.4% PPZHCl were 100, 99.4, and 88.7%, respectively. The relative standard deviation (RSD) values from triplicate analysis of these formulations ranged from 0.7 to 6.7%. The PPZHCl content from 9 manufactured TTDs, 3 for each formulation type, were analyzed in triplicate and produced RSD values ranging from 0.7–6.8%. These results indicate that the formulation extraction presented could be used to evaluate the PPZHCl content in TTDs prior to field use. The use of a small bore LC column reduced the amount of solvents consumed and hazardous waste generated, compared to sample analysis that uses a more conventional analytical LC column.

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Propionylpromazine hydrochloride (PPZHCl) is used by cattle and swine producers to reduce animal stress during transportation from farms to slaughterhouses (1), while commercially available Tranvet® tablets that contain PPZHCl are used by veterinarians to sedate dogs. To evalulate PPZHCl residue levels in swine tissues, a number of chromatographic methods including high-performance liquid chromatography (HPLC; 1), gas chromatography (2), and thin-layer chromatography (3, 4) have been developed. Recently, the U.S. Department of Agriculture (USDA) evaluated the use of PPZHCl with foothold traps to reduce post capture trauma. Such traps are frequently used by the Wildlife Services (WS) program to capture coyotes that prey on livestock. In efforts to release itself from the trap, the animal itself may inflict other types of trauma including broken teeth, tongue and gum lacerations (5), along with chewing and gnawing of the restricted appendage (6). Incorporating a tranquilizer delivery mechanism in a leg hold trap may reduce the amount of self inflicted trauma on a restrained animal.

Balser (6) tested such a device by attaching to a leg hold trap a chewable apparatus consisting of semi-rotten cloth, petroleum jelly, and the sedative diazepam (7-chloro-1,3-dihydro-1-methyl-5-phenyl-2H-1,4-benzodiazepin-2-one) sealed with a bees wax and paraffin mixture. The idea was that a restrained animal, in an effort to free itself, would chew on this tranquilizer trap device (TTD) and ingest the sedative. Balser observed that captive animals chewed on the delivery device. Diazepam ingestion caused drowsiness and the reduction or absence of biting which reduced the number of self-inflicted wounds. However, diazepam, being on the Drug Enforcement Administration list of controlled substances, was never authorized for use with TTD. Laboratory experiments (7) and field studies (8) found PPZHCl to be a favorable substitute for diazepam. Linhart (8) noted that during a 24-h check period, 86% of captive animals restrained by control traps not equipped with a tranquilizer device exhibited some type of foot damage, while only 10–25% of the animals

Figure 1. Tranquilizer trap devices (TTDs).

caught in leghold traps equipped with PPZHCl-containing TTD exhibited foot damage.

To aid WS in the development of TTD, the National Wildlife Research Center (NWRC) obtained an Investigational New Animal Drug Application (INADA) from the U.S. Food and Drug Administration for PPZHCl. In a study, Savarie and Roberts (7) reported that 600 mg PPZHCl produced the quickest and most effective tranquilizing affect on test coyotes. With this, 2 types of TTD were evaluated by WS. The first device contained 600 mg PPZHCl and 4 mL Vaseline in a dart balloon (Figure 1A). The inlet is tied in a knot and the balloon is then wrapped with cheese cloth and the device is sealed with paraffin. The second TTD uses a McBride device (Livestock Protection Co., Alpine, TX) that holds 2.5 mL Karo dark corn syrup or K-Y Jelly formulation containing 600 mg PPZHCl (Figure 1B). To evaluate the PPZHCl contents in these TTD formulations, we developed an HPLC assay method. To minimize the quantity of solvents consumed and hazardous waste generated by these analyses, we used a small bore LC column (15 cm × 2.0 mm id) with a mobile phase flow rate of 0.30 mL/min.

Experimental

Reagents

The following chemicals were used with the assay methods presented: acetonitrile (ACN; high purity, Burdick and Jackson, Muskegon, MI); deionized water (Milli-Q System, Millipore, Bedford, MA); hexane (reagent grade, Fisher Scientific, Denver, CO); HCl (36% w/w; ACS Certified Plus, Fisher Scientific); 0.2M heptanesulfonic acid pre-mixed solution (Alltech Associates, Inc., Deerfield, IL).

A 0.05N $HCl_{(aq)}$ solution was prepared by diluting 4 mL HCl (36% w/w) to 1000 mL with deionized water. An ion-pairing (IP) solution was prepared by combining 25 mL of the heptanesulfonic acid premix with 1000 mL deionized water to produce a 5 mM heptanesulfonic acid solution. A diluent was prepared by combining 350 mL ACN with 150 mL of the IP solution.

Instrument Parameters

Sample analysis was performed using a Hewlett Packard liquid chromatograph (Model 1090M, Palo Alto, CA) equipped with a diode array ultraviolet (UV) detector. Injection volumes of 1 µL were eluted through a small bore C_{18} column (ODS/H, 15 cm × 2.0 mm, 5 µm particle size, Keystone, Bellefonte, PA) heated to 40°C. The mobile phase was ACN–IP solution (85 + 15) at a flow rate of 0.30 mL/min. Component mixing of the mobile phase was done by the liquid chromatograph. Absorbance data were collected at a wavelength of 280 nm. After each set of analyses, the LC column was washed using a set of mobile phase gradients (Figure 2).

Assay of PPZHCl Technical Material

An assay method was developed and validated to assess the purity of PPZHCl technical material (Diamond Laboratories, Des Moines, IA) used to prepare formulations. A PPZHCl reference material (99%) was obtained from Crescent Chemical Co., Inc., Hauppauge, NY. A concentrated stock solution from each source was prepared by accurately weighing 10 mg of the technical or reference PPZHCl material and quantitatively transferring to a 10 mL volumetric flask. The solid was dissolved and diluted to volume with water producing a PPZHCl concentration of 1000 µg/mL. Working solutions for each were prepared by transferring a 0.500 mL volume of the concentrated stock solution into a 10 mL volumetric flask, diluting to volume with the diluent and mixing well. The PPZHCl concentration of each solution was ca 50 µg/mL.

Injection aliquots of 1 µL from each solution were chromatographed using the presented instrument parameters. The purity of the PPZHCl technical material was determined by comparing the chromatographic response from its working solution with the response of the working solution from the PPZHCl reference material.

15 % IP		0 % IP		0 % IP		0 % I P		0 % IP
85 % ACN	→	85 % ACN	→	95 % ACN	→	5 % ACN	→	15 % ACN
0 % Water	*(5 min)*	15 % Water	*(0.5 min)*	5 % Water	*(5 min)*	95 % Water	*(5 min)*	85 % Water
		(2 min)		*(10 min)*		*(10 min)*		*(7.5 min)*

Figure 2. Mobile gradients for LC column washout.

Table 1. In-house formulation preparations

Matrix	Amount, g	PPZHCl amount, g	PPZHCl content, % w/w
	High-level formulations		
Vaseline	1.25	0.792	38.8
Karo syrup	1.49	0.537	26.5
K-Y Jelly	1.36	0.671	33.0
	Mid-level formulations		
Vaseline	3.17	0.615	16.2
Karo syrup	2.72	0.602	18.1
K-Y Jelly	2.00	0.613	23.5
	Low-level formulations		
Vaseline	3.17	0.305	8.78
Karo syrup	2.72	0.314	10.3
K-Y Jelly	2.00	0.310	13.4

Assay of PPZHCl Formulations

(a) *Preparation of in-house formulations.*—For each control matrix, 3 PPZHCl formulations were prepared (Table 1) by: (*1*) recording the weight of an empty 10 mL graduated centrifuge tube; (*2*) drawing up the control matrix into a glass pipet, dispensing the appropriate amount into the preweighed tube, and recording the weight; (*3*) adding the specified amount of PPZHCl technical material to the centrifuge tube, recording the final weight, and mixing with a spatula until the active ingredient is uniformly dispersed throughout the matrix. The Vaseline matrix was melted in boiling water (ca 100°C) prior to use.

The resulting Vaseline and Karo formulations were viscous suspensions. However, separation of the Karo syrup and PPZHCl occurred when this formulation sample was not used for 7 days. The K-Y matrix appeared to dissolve the PPZHCl producing a formulation sample that was a dark brown transparent gel.

(b) *Standard preparation.*—A 10 mg sample of PPZHCl reference material was accurately weighed and placed in a 10 mL volumetric flask. This material was dissolved in and diluted to volume with water then mixed. The PPZHCl concentration of this stock solution was 1000 μg/mL. A 200 μg/mL PPZHCl working standard was prepared by transferring a 2.00 mL aliquot from the stock solution to a 10 mL volumetric flask, diluting to volume with diluent, then mixed.

(c) *Vaseline formulation extraction.*—A 100 mg formulation sample was quantitatively transferred to a tared 45 mL glass centrifuge tube and weighed. To aid the extraction process, the formulation sample was smeared on the inside wall in the conical portion of the centrifuge tube. A 10 mL aliquot of hexane was added to the tube which was mixed on a Vortex mixer then placed in a water bath and sonicated for 5 min. Having dispersed the sample, a 10 mL aliquot of the 0.05N $HCl_{(aq)}$ solution was added to the tube which was mixed on a Vortex mixer and centrifuged (835 × *g*) for 2 min. Using a stainless steel N-Evap™ needle, ca 4″ in length, attached to a 10 mL plastic syringe, the aqueous layer was carefully removed from the tube and transferred to a 50 mL volumetric flask. The hexane layer was extracted with a second 10 mL aliquot of the acid solution. Having combined both aqueous extracts in the same flask, the sample was diluted to volume using the acid solution and thoroughly mixed. A 2.00 mL aliquot from the acid solution was transferred to a 10 mL volumetric flask, diluted to volume with diluent, and mixed. Due to the photosensitivity of PPZHCl, an aliquot from the resulting solution was filtered through a 0.45 μm PTFE syringe fil-

Table 2. Regression analysis of analyte response versus PPZHCl concentration

	x/y plot		log(x)/log(y) plot	
PPZHCl concn range, μg/mL	r^2	Intercept	r^2	Slope
10.1 to 1010	1.0000	2.096	0.9997	1.004

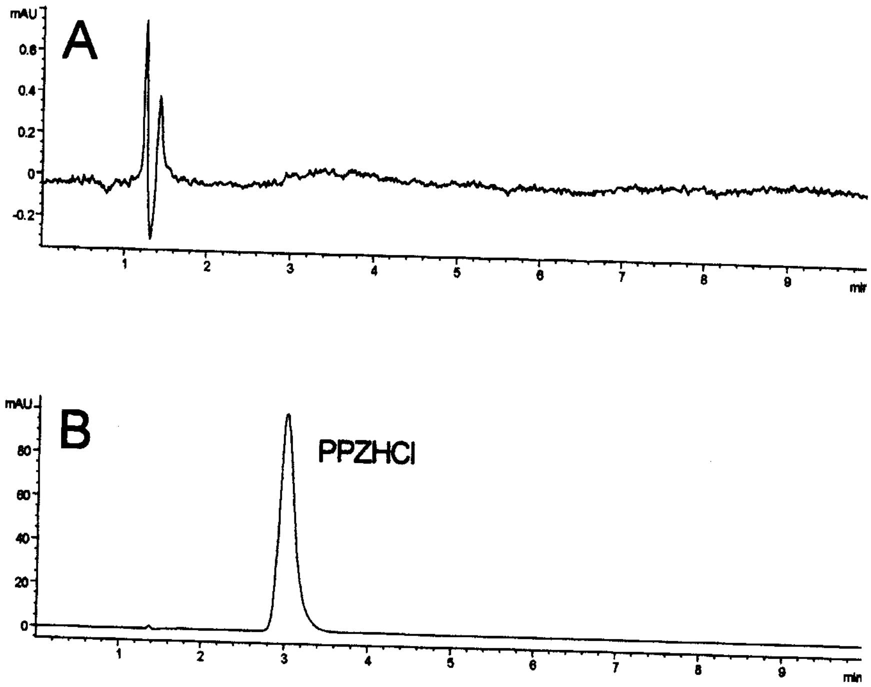

Figure 3. Chromatograms of (A) diluent, and (B) calibration solution at 212 μg/mL.

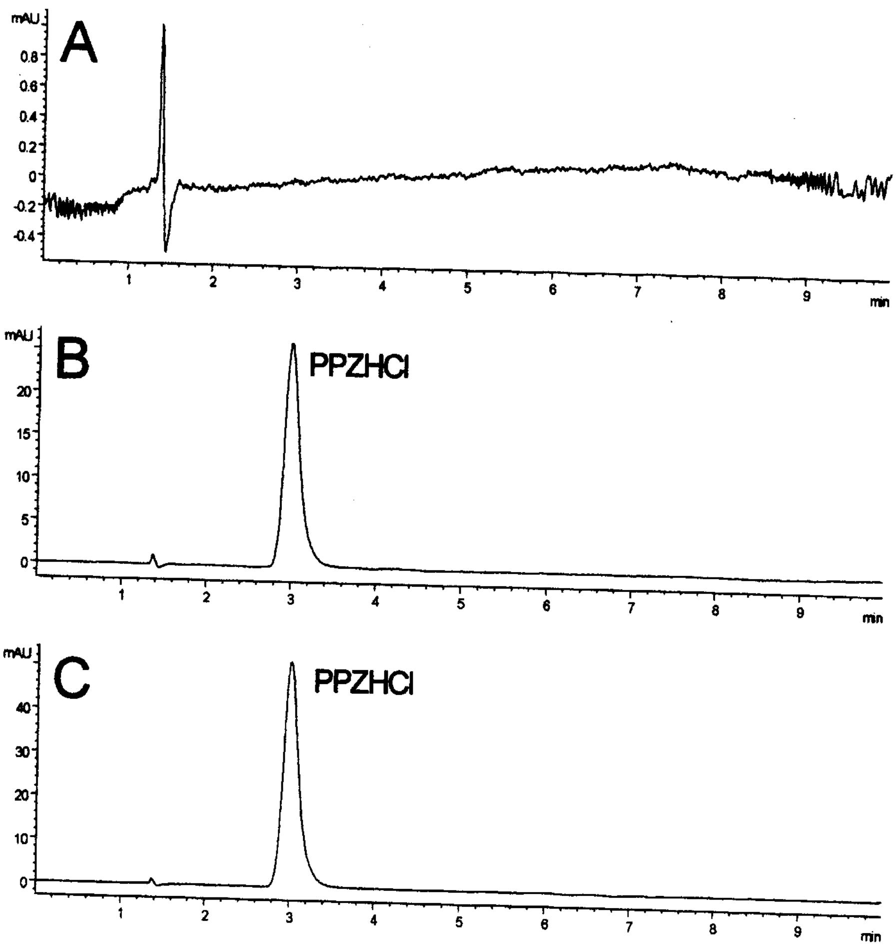

Figure 4. Chromatograms of (A) a blank Vaseline control sample extract, (B) a 16.4% w/w Vaseline in-house formulation extract, and (C) a Vaseline TTD formulation extract.

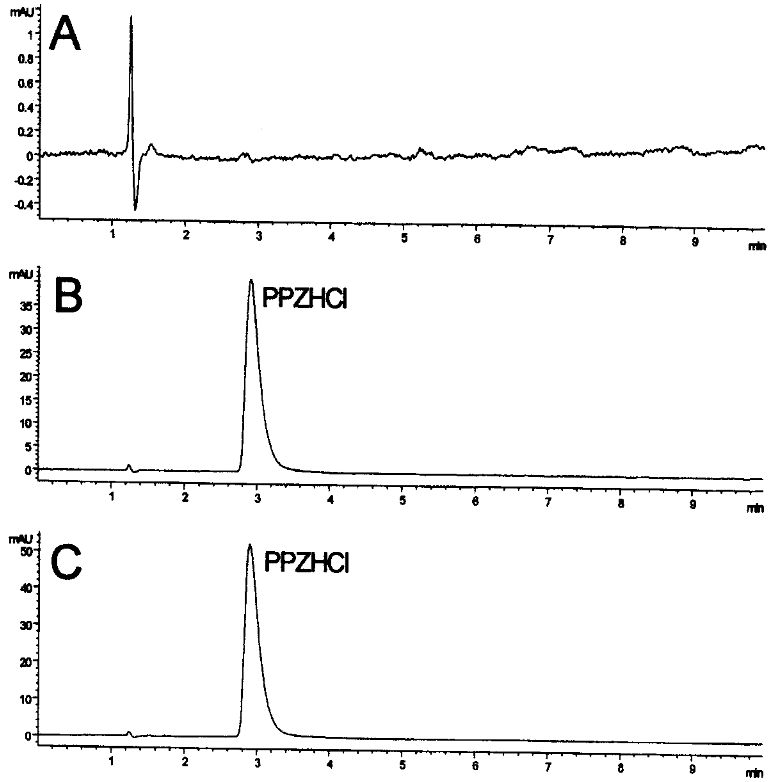

Figure 5. Chromatograms of (A) a blank Karo dark syrup control extract, (B) an 18.1% w/w Karo dark syrup in-house formulation extract, and (C) a Karo dark syrup TTD formulation extract.

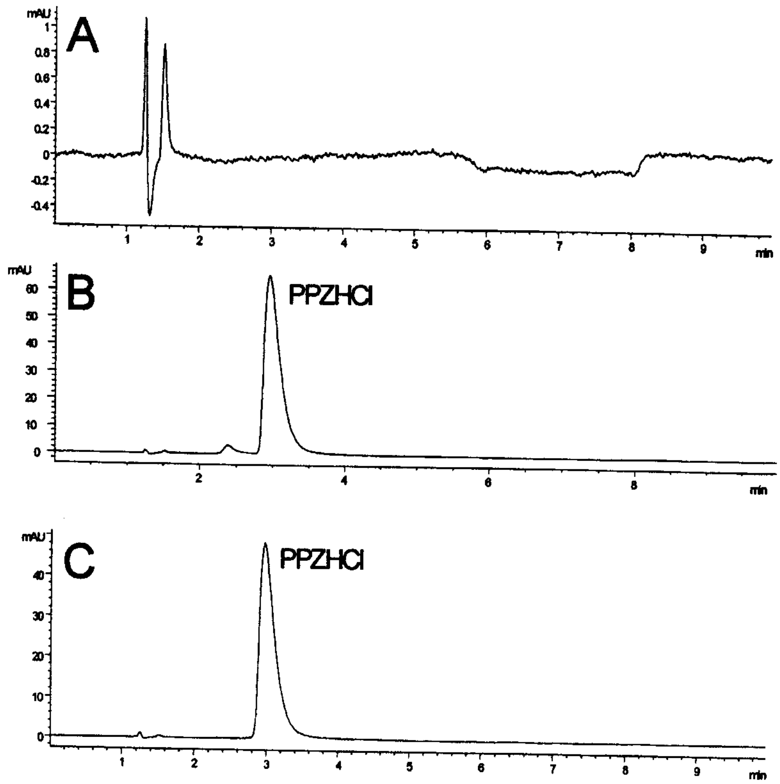

Figure 6. Chromatograms of (A) a blank K-Y Jelly control extract, (B) a 23.8% w/w K-Y Jelly in-house formulation extract, and (C) a K-Y Jelly TTD formulation extract.

Table 3. PPZHCl recovery and reproducibility from in-house formulations

Formulation matrix	PPZHCl level, % w/w	Analyte recovery ($n = 3$)		
		Mean, %	s, %	RSD, %
Vaseline	38.8	104	2.0	1.9
	16.2	92.9	6.2	6.7
	8.78	90.2	1.9	2.1
Karo dark syrup	26.5	97.7	1.7	1.8
	18.1	99.3	3.1	3.1
	10.3	106	2.4	2.3
K-Y Jelly	33.0	100	3.0	3.0
	23.5	99.4	0.7	0.7
	13.4	88.7	1.4	1.6

ter (Scientific Resources Inc., Eatontown, NJ) into an amber glass LC vial which was sealed with a crimp cap.

(**d**) *Karo and K-Y formulation extractions.*—A 100 mg formulation sample was quantitatively transferred to a tared 45 mL glass centrifuge tube and weighed. To aid the extraction process, the formulation sample was smeared on the inside wall in the conical portion of the centrifuge tube. A 10 mL aliquot of the 0.05N $HCl_{(aq)}$ solution was added followed by mixing on a Vortex mixer. The tube was placed in a water bath and sonicated for 5 min, after which the sample solution was transferred to a 50 mL volumetric flask. The centrifuge tube was further rinsed with three 10 mL portions of the acid solution which were also added to the 50 mL flask. Having completely dissolved the sample, the resulting solution was diluted to volume with the acid solution and mixed on a Vortex mixer. A 2.00 mL aliquot from the acid solution was transferred to a 10 mL volumetric flask, diluted to volume with diluent, and mixed. An aliquot from the final solution was filtered through a 0.45 μm PTFE syringe filter into an amber glass LC vial which was sealed with a crimp cap.

(**e**) *Manufactured TTD evaluations.*—The contents from 9 TTDs produced at the Pocatello Supply Depot (Pocatello, ID) were extracted according to the procedures described for each formulation. Three TTD were McBride devices containing a K-Y Jelly formulation; 3 other McBride devices contained a Karo syrup formulation. The extraction procedure for the Karo and K-Y Jelly in-house formulations was used to determine the PPZHCl content in these devices. Prior to sampling, the contents in each device were mixed using a metal spatula. The 3 final TTDs contained a Vaseline formulation in a dart balloon. To ensure sample homogeneity, each dart balloon was placed in a beaker of hot tap water for 2 min. The contents in each balloon were mixed between the thumb and forefinger for 2 min prior to sampling. The PPZHCl content in

Table 4. Observed PPZHCl content in manufactured TTDs

Matrix	TTD device	PPZHCl target content, % w/w	Sample	Observed content ($n = 3$)		
				Mean (% w/w)	s (% w/w)	RSD, %
Vaseline	Dart balloon	16.0	1	20.5	0.58	2.8
			2	24.9	1.6	6.4
			3	26.4	0.49	1.9
	In-house formulation	16.4	—	16.9	0.12	0.7
Karo dark syrup	McBride	18.0	1	17.6	1.2	6.8
			2	16.1	0.12	0.7
			3	15.7	0.45	2.9
	In-house formulation	18.1	—	18.5	0.9	5.2
K-Y Jelly	McBride	23.0	1	19.9	1.0	5.0
			2	19.8	0.32	1.6
			3	18.9	0.79	4.2
	In-house formulation	23.8	—	22.3	0.5	2.6

each of these devices was determined using the methodology for the Vaseline in-house formulations.

PPZHCl Quantitation

Aliquots from sample and standard solutions were analyzed by HPLC. The PPZHCl content in each formulation sample was determined by comparing the UV response from the final sample solution to the response from the working standard solution. For all formulation samples analyzed, the PPZHCl content was calculated with the equation:

$$\text{PPZHCl content, \% w/w} = \frac{\text{area}_{\text{sample}}}{\text{area}_{\text{std.}}} \times$$

$$\text{std. conc., }\mu\text{g/mL} \times \frac{10.00\text{ mL}}{2.00\text{ mL}} \times$$

$$\frac{50.00\text{ mL}}{\text{sample wt., g}} \times \frac{1\text{g}}{10^6\ \mu\text{g}} \times 100$$

Results and Discussion

Response Linearity

Eight calibration standard solutions with PPZHCl concentrations ranging from 10.1–1010 μg/mL were prepared, and each solution was chromatographed 4 times. A linear regression was performed on the data set that consisted of the mean peak response for each standard solution along with the corresponding PPZHCl concentration. The regression results (Table 2) exhibited a linear and directly proportional relationship between analyte peak response and PPZHCl concentration. Therefore, single-point calibrations were valid over the PPZHCl concentration range evaluated.

Diluent injections (Figure 3A) indicated no chromatographic interferences having the same retention time as PPZHCl. The purity of the technical material was evaluated using a 49.5 g/mL PPZHCl standard solution, while PPZHCl quantitations of in-house formulations and TTD contents were calculated using a 212 g/mL standard solution (Figure 3B).

Assay of PPZHCl Technical Material

The purity of seven 10 g samples from the PPZHCl technical material were determined using the PPZHCl reference material. The mean purity of the PPZHCl technical material was found to be 97.0% with a relative standard deviation (RSD) of 1.2%.

In-House Formulations and TTD Evaluations

(**a**) *Matrix interference and method limit of detection.*—Control samples from each formulation matrix were extracted using the presented method. The resulting chromatograms (Figures 4A, 5A, and 6A) indicated no interfering responses having the same retention time as PPZHCl. The method limit of detection (MLOD) was defined as the concentration of PPZHCl in a formulation sample that would produce a response that was 3 times the baseline noise. For each formulation, the MLOD was calculated using the height of the baseline noise observed in the control chromatograms and the height of the analyte response from a control matrix fortified at the PPZHCl target level (Figures 4B, 5B, and 6B). The MLOD for the Vaseline, Karo syrup, and K-Y Jelly formulations were 0.09, 0.09, and 0.08%, respectively.

(**b**) *Recovery and reproducibility.*—Table 3 contains the PPZHCl recovery data for the in-house formulations prepared at each PPZHCl level. Mean analyte recoveries for the Vaseline in-house formulations ranged from 90.2–104%, with RSD values ranging from 1.9–6.7%. For the Karo syrup in-house formulations, PPZHCl recoveries ranged from 97.7–106% with RSD values ranging from 1.8–3.1%. Finally, the K-Y Jelly in-house formulations exhibited recovery values of 88.7–100%, with RSD values ranging from 0.7–3.0%.

Table 4 contains the observed PPZHCl content in the TTD formulations along with another set of in-house formulations that were concurrently assayed. Figures 4C, 5C, and 6C are example chromatograms of the PPZHCl extracts from each TTD type.

Conclusions

Regression analysis of the linearity data indicated that single-point calibrations were valid over the PPZHCl concentration range evaluated. The validated method used to evaluate the PPZHCl technical material produced a precise and acceptable purity value (>97%) for this material. The recovery and repeatability data from the analysis of the in-house formulations showed that the method could accurately and precisely determine PPZHCl content in these samples. The method also produced precise PPZHCl results from the 3 samples that were assayed for each TTD type. Therefore, the extraction method could be used to evaluate and verify the PPZHCl content of manufactured TTDs. In addition, using a small bore LC column during sample analysis provided a 2-fold cost savings. The use of a lower mobile phase flow rate (0.30 mL/min) consumed a lesser amount of chemicals and produced less hazardous waste. Such costs would be greater for an assay method that uses more conventional analytical LC columns which require a higher mobile phase flow (1 mL/min) to produce comparable results.

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